



US009486514B2

(12) **United States Patent**
Deora et al.(10) **Patent No.:** US 9,486,514 B2
(45) **Date of Patent:** *Nov. 8, 2016(54) **BORDETELLA OUTER-MEMBRANE PROTEIN ANTIGENS AND METHODS OF MAKING AND USING THE SAME**(71) Applicant: **Wake Forest University Health Sciences**, Winston-Salem, NC (US)(72) Inventors: **Rajendar K. Deora**, Winston-Salem, NC (US); **Meenu Mishra**, Winston-Salem, NC (US); **Neelima Sukumar**, Winston-Salem, NC (US)(73) Assignee: **Wake Forest University Health Sciences**, Winston-Salem, NC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **15/014,665**(22) Filed: **Feb. 3, 2016**(65) **Prior Publication Data**

US 2016/0144015 A1 May 26, 2016

Related U.S. Application Data

(63) Continuation of application No. 14/498,537, filed on Sep. 26, 2014, now Pat. No. 9,283,268, which is a continuation of application No. 12/680,823, filed as application No. PCT/US2008/012051 on Oct. 23, 2008, now Pat. No. 8,877,201.

(60) Provisional application No. 60/982,513, filed on Oct. 25, 2007.

(51) **Int. Cl.****C07K 14/235** (2006.01)
A61K 39/02 (2006.01)

(Continued)

(52) **U.S. Cl.**CPC **A61K 39/099** (2013.01); **C07K 14/235** (2013.01); **C07K 16/1225** (2013.01);

(Continued)

(58) **Field of Classification Search**CPC A61K 39/99; C07K 14/235
USPC 424/253.1, 254.1, 164.1, 242.1
See application file for complete search history.(56) **References Cited**

U.S. PATENT DOCUMENTS

3,873,691 A	3/1975	Kasuga et al.
4,016,253 A	4/1977	Switzer et al.
4,225,583 A	9/1980	Switzer et al.
4,888,169 A	12/1989	Brown et al.
5,545,670 A	8/1996	Bissbort et al.

(Continued)

FOREIGN PATENT DOCUMENTS

WO WO 2005/032584 A2 4/2005

OTHER PUBLICATIONS

Weir, D. M. Handbook of experimental immunology, chapter 1, Immunochemistry, sections 8.14-8.15, pp. 1-4, Oxford: Blackwell Scientific 1986.*

(Continued)

Primary Examiner — Albert Navarro*Assistant Examiner* — Ginny Portner(74) *Attorney, Agent, or Firm* — Myers Bigel & Sibley, P.A.(57) **ABSTRACT**

An isolated protein or peptide selected from the group consisting of *Bordetella* colonization factor A (BcfA) protein and antigenic fragments thereof is described, along with an isolated nucleic acid encoding the same, antibodies that bind to the same, methods of producing an immune response in a mammalian subject in need thereof by administering the proteins, peptides or antibodies, and pharmaceutical compositions comprising the same.

15 Claims, 4 Drawing Sheets

LTLGAVRTHPGTVVVTGKTGPGAKVRIDFPDGTGFDVVAGNGGDFTVASKGDVTA
SGPIVAIARDDGRESPRRTVQYDDRVNGGSGAPTVVLHTDGTNGRVTSGKGRPG
DTIRVDPDGTKEVVAGPDGTYRVTSDRDMTAGDITVSGTDAKGNVGPPVKRPYHD
IFVPVPTVEVATDSSGRVTSGKATPRAKVVKDFPGGTSKTVTADADGRYRATSD
GDVPGGDIVVTQTMGAAGKPVRRPYDVTAPTPMKVTDMSRTDGNSGVVTG
TVGGSTVTVTFPDGTTAGTTANDRGKYTVTSTADIPAGPIRVSARGPRNQQGSATDH
YLDWTKQTLGGKIRLLRPVARLLLSPGSMTYTEIAKSFDGSSLGDIVARFEPANG
APPQTAALLAAIKLHDPNYRLESNKMFYIYDTMNSDPYNRVPNGDYPVTLVLEDKAT
GAREATTMVLKVTVGTYGKAPVVPAGNGVLGTGPGPSLGGSLIGGEGGLGS
(SEQ ID NO: 3)

- (51) **Int. Cl.**
C07K 16/12 (2006.01)
G01N 33/569 (2006.01)
A61K 39/00 (2006.01)
- (52) **U.S. Cl.**
CPC ... *G01N33/56911* (2013.01); *A61K 2039/505* (2013.01); *A61K 2039/55505* (2013.01); *A61K 2039/575* (2013.01); *G01N 2333/235* (2013.01)

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,798,103 A	8/1998	Mooi	
5,877,298 A *	3/1999	Fahim	A61K 39/099 530/412
6,036,953 A	3/2000	Ryan et al.	
6,582,705 B1	6/2003	Gueirard et al.	
7,049,423 B2	5/2006	Ryan	
7,101,987 B2 *	9/2006	Vicari	C07K 16/24 530/350
7,151,082 B2 *	12/2006	Tracey	A61K 39/395 424/130.1
7,163,685 B2 *	1/2007	Diamond	A61K 39/245 424/199.1
7,211,411 B2 *	5/2007	Neefe	A61K 38/162 424/192.1
7,270,816 B2 *	9/2007	Timans	C07K 14/54 424/139.1
7,479,283 B1	1/2009	Novotny	
7,659,388 B2	2/2010	Oh et al.	
8,507,201 B2 *	8/2013	Cockerill, III	C12Q 1/689 435/6.1
8,669,091 B2	3/2014	Gentschev et al.	
8,877,201 B2 *	11/2014	Deora	A61K 39/099 424/164.1
9,283,268 B2 *	3/2016	Deora	A61K 39/099
2004/0029129 A1 *	2/2004	Wang	C07K 14/195 435/6.18
2004/0115210 A1	6/2004	Timmerman	
2006/0121450 A1	6/2006	Miller et al.	
2007/0116711 A1	5/2007	Castado et al.	
2008/0081770 A1	4/2008	Oh et al.	
2010/0028379 A1	2/2010	Tucker et al.	

OTHER PUBLICATIONS

- Amara, A et al, Neuroscience Letters, Feb. 13, 1995, vol. 185(3), pp. 147-150, Molecular detection of methionine in rat brain using specific antibodies.*
- Ellis, Chapter 29, pp. 568-575, Vaccines, 1991.*
- Boslego, Chapter 17, pp. 211-223, Gonorrhea vaccines, Vaccines and Immunotherapy 1990.*
- Dolby JM et al. The effect of the antigen which elicits the bactericidal antibody and of the mouse-protective antigen on the growth of *Bordetella pertussis* in the mouse brain. J. Hyg. Camb. 1975; 74: 71-83.
- Sukumar N et al. Differential Bvg phase-dependent regulation and combinatorial role in pathogenesis of two *Bordetella* paralogs, BipA and BcfA. Journal of Bacteriology. May 2007; 189(10): 3695-3704.
- Smith IM et al. Immunisation of pigs against experimental infection with *Bordetella bronchiseptica*. Veterinary Record. May 22, 1982; 110(21): 488-494.
- Pittman M. Letter: Protective activity of whooping-cough convalescent serum and serum-IgA level in mice infected with *Bordetella pertussis*. Lancet. Jul. 17, 1976; 2(7922): 156.
- Weir DM. Handbook of experimental immunology, chapter 1, Immunochemistry, sections 8.14-8.15, pp. 1-4, Oxford: Blackwell Scientific, 1986.
- Mattoo S et al. Regulation of type III secretion in *Bordetella*. Molecular Microbiology; 2004; 52(4): 1201-1214.
- NCBI Accession No. NP_886662, Adhesin *Bordetella bronchiseptica* RB50, pp. 1-3, deposited by Mattoo et al. 2004.
- Parkhill J et al. Comparative analysis of the genome sequences of *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella bronchiseptica*. Nature Genetics. Sep. 2003; 35(1): 32-40.
- UniProt Accession No. Q7WR47, Oct. 1, 2003, one page, *Bordetella bronchiseptica* BB0110 putative adhesin.
- Sebaihia M et al. Comparison of the genome sequence of the poultry pathogen *Bordetella avium* with those of *B. bronchiseptica*, *B. pertussis*, and *B. parapertussis* reveals extensive diversity in surface structures associated with host interaction. Journal of Bacteriology. Aug. 2006; 188(16): 6002-6015.
- Amara A et al. Molecular detection of methionine in rat brain using specific antibodies. Neuroscience Letters. Feb. 13, 1995; 185(3): 147-150.
- Sukumar N et al. Differential Bvg phase-dependent regulation and combinatorial role in pathogenesis of two *Bordetella* paralogs, BipA and BcfA. Journal of Bacteriology. May 2007; 189(10): 3695-3704.
- Mattoo S et al. Mechanisms of *Bordetella* pathogenesis. Front Biosci. Nov. 1, 2001; 1(6); pp. E168-E186.
- Friedman LE et al. Short communication, Characterization of *Bordetella bronchiseptica* strains using phenotypic and genotypic markers. Veterinary Microbiology. 2006; 117: 313-320.
- Fernandez J et al. Constitutive expression of bvgR-repressed factors is not detrimental to the *Bordetella bronchiseptica*-host interaction. Research in Microbiology. 2005; 156: 843-850.
- Vergara-Irigaray N et al. Evaluation of the role of the Bvg intermediate phase in *Bordetella pertussis* during experimental respiratory infection. Infect. Immun. Feb. 2005; 73(2): 748-760.
- Finn TM and Stevens LA. Tracheal colonization factor: a *Bordetella pertussis* secreted virulence determinant. Molecular Microbiology. 1995; 16(4): 625-634.
- Leininger E et al. Inhibition of *Bordetella pertussis* filamentous hemagglutinin-mediated cell adherence with monoclonal antibodies. FEMS Microbiology Letters. 1993; 106: 31-38.
- Vergara-Irigaray N et al. Evaluation of the role of the Bvg intermediate phase in *Bordetella pertussis* during experimental respiratory infection. Infection and Immunity. Feb. 2005; 73(2): 748-760.
- NCBI database accession No. NP_886662 "Putative adhesion [Bordetella bronchiseptica RB50]", Jun. 7, 2007, 2 pp.
- Sukumar N et al. Differential Bvg-phase-dependent regulation and role in pathogenesis of two *Bordetella* paralogs. Mid-Atlantic Microbial Pathogenesis Meeting, Feb. 11-13, 2007, Wintergreen, VA. Abstract.
- Sukumar N et al. Differential Bvg-phase-dependent regulation and role in pathogenesis of two *Bordetella* paralogs, BipA and BcfA. ASM 107th General Meeting, May 21-25, 2007, Toronto, Canada. Abstract.
- Sukumar N et al. Active and passive immunizations with *Bordetella* colonization factor A protect mice against respiratory challenge with *Bordetella bronchiseptica*. Infection and Immunity. Feb. 2009; 77(2): 885-895.
- Zhao Z et al. Immunogenicity of recombinant protective antigen and efficacy against intranasal challenge with *Bordetella bronchiseptica*. Vaccine. 2009; 27: 2523-2528.
- International Search Report and Written Opinion, PCT/US2008/012051, mailed Aug. 19, 2009.

* cited by examiner

GTGAAGCAAGCCATCCACGCCGTTGCCCTGCCATGATGCGCTCGCACGAGTCGGCGTGTCCATCGG
CGCCGCGGCCGCCGCGCTGGCTGGCGCTTGACGCTGCAAACCGTGGCGCCGGCATTGCCCAGGGG
GCGCCGTCTTCTCCGCCCGGCCGCCGCGCAGGCCGATGCCAGGATGCCGCCACAGCGCGATGCTGCC
GTCGCGCAGACGGCGGCCAATTGGCGAACGCCAGGCTGCCGTTGCCGCCCTGGCGCGCTGGAC
GGCGACTTGCTGAAAGGACAGGCCAGGCCAATGAGTTGCTGCAGGAAGGGTGCGCCCTGGCC
AACCAAGACTGAATTGCCGTTCCCTGCCGCCGGTGCAGGCCAGGCGGGTGAATTATGACTTTCGAACAAAGGAC
CTGTCGTTGGATCTTCGTAACCATCGACGAAGTGCATGCCGCCAGCGCGACC CGCTTGCTGCAACTG
AGCGGCCACAATCGCAATCATCGTCCACCGTCAACGGTGGCGTGGTGTGCGCCATGCCCTGAACCAG
CACATGCCGTGGCGCAACGCATTCTGATTACGAGTCGCCAAGAACCATCTGCCGCCCTCGCTG
GGCGAGAGGTATTGCCCGCAGTTACGCTGTATGCCAACGTCTACGCCCCATGTCGGGATGGAAA
GCGGCCAACGCCGCCAGCGCOCGCGAACAGGCCGCCCTGGCGTGGACGTTGCCGTGCCCTGCAA
CCCGAGGCCGTGCCCTGCCCTGGCAATCAAGGCCAGTATTCCGCTGGAGCGGCCGCCGTGGATTAC
TTCGACAACGCCGTCCGCAGCGCAATGCCGCCGCTATAAGTACGCCGTTGAGTACCGGCCGTGCCG
TTGGTGGCGGTGGCGCTGGAACAGACCAAGGTGCTGCCGCCGCGCCAGACCACTGTGAGCTTGC
GTCATCTCAGCCTGGCGAGCCCTGTCCAGGCAGTTGCCGACCAGTCCGGCGGCTTGACTG
CAGGCCCGCATGGCGAATTGTCGAGCGTAAAACCGCATCGTGTCTCAGACGCCGCCAACAGT
GTGTTGCCGTGACGATCGCGCGTCGATACCGATCCGCAACCGGGGATCACGTAACCGCGTC
ACCGAGCCGGGGCGCAGGTCAAGCTGGCGAACATGGCGAACGTCGTTGCCGAGGCCGATGGC
AGCGGAACCTACCGAGCGACGTCGGCGCGACATGGTGGCGCCGGCTCGCGAACAGAAC
CGTCATGCCGACCGTAGCCGGAAAGTCACGCCACATTACGTTGATGTCGCCGCAAGGGCGAGGTACCG
CTGACGCTGGCGCTGCGCACGCATCTGCCACGGCCTCGTACCGTGACCCGCAAGACCGGGCCT
GGCGCCAAGGTGCGCATCGATTTCCGACGGTACGGTACGTTGATGTCGCCGCAATGGGGCGAT
TTCACGGTCGCTCGAACAGGCGATGTGACGGCAGCGGCCGATCGTGGCGATTGCCGCGATGACGAC
GGCGGGAAAAGCCCCCGCGTACTGTCAGTACGACAGGGTCAATGGCGGTGGCTCGGGCGCG
ACGGTGGTGTGCAACCGACGGCACCAACGGTACGGTACGGTACGGCAAGGACGCCGGCGAT
ACGATCAGGGTGGACTTCCCACGGCACCCACCAAGGAGGTGGTGGCGGGCCGACGGCACCTACCGC
GTCACGTCCACCGCGACATGACGGCGGGCGACATAACGGTGTCCGTAACGATGCCAACGGCAACGTG
GGTGGTCTGTCAGCGTCCCTACACGACATTCTCGTGCCGTGCCGCCACCGTGGAGGTGGCGACC
GACTCGTCAGCGGCCGGTCACGGTACGGTACGGCAAGGCCACGCCGCCGCCAACGGTCAAGGTG
CCGGCGGGGACGCTCAAGACCGTCACCGCGACGCCACGGCGCTATCGCGCACCTCGGATGCCGAC
GTGCGCTGGGGCGACATCGTGTACCGCAGACCGGGATGCCGGCGCTGCCGCAAGCGGTGCGTCGA
CCGTATGTCGATACGGTGGCGCCGACGCCGATGAAAGTACCGATCGACAGCATGCCACGGACGGCAAC
ACGGCGTGGTACGGTACGGCTACACGGTCCGCGGCTCCACGGTACGGTACGGTACCGTCCCGACGGC
ACGACCGGGTACCCGCCAATGACCGAGGAAATACACGGTAACGTCGACCGGCCGACATTCCG
GGTCCGATCCCGTCAGCGCCGGGACGCCGCAACCGCAGGGCAGCGCACGGGACCGGACATTACCTCGAT
GGTGGACCAAGCAGACGCTGCTGGCGCAAGATTGCCCTCTCCGCCGGTCCGAGGCTGTTGCTG
AGCCCGGGCAGCATGACATATACGAAATGCCAACGCTGATGCCAGTTGCTCGACGGCATCGT
GCAACGGTTCGAGCCGGCAAACGGAGCACCGCCGAGACGCCGGCTGCTGCCGCGATCAAGCTGCAC
GATCCAAATTATCGGCTGGAGTCCAACAAAGATGTTCATCTATCTCGACACCATGAACAGCGACCCGTAC
AACCGTGTCCCAACGGCGATTATCCGTCAGGCTGGTTCTCGAGGACAAGGCCACCGGGCGGGAG
GCGACCACCATGGTCTGAAGGTGACCGCGAGTACCTATGCCAACAGCCCCGGTGTCCCCGGCGAAT
GGTGTGCTGGCACGGGGCCGGCCGTCGTTGGCGGCAGTCTGATCGGTGGCAGGGCGGGCTG
CTGGGAAGCTGA (SEQ ID NO:1)

FIG. 1

MKQAIHAVAFRHALARVGRVHRRGAAALAGVLTIQTVAFAQGAPSFSARPAQA
DRQDAADSAMLRVAQTARQLAQRQAAGSRASARVDGDLLKGQAEAQANEQQEGVRL
ANQTELPFLRRLQGGVNYDFSNKDLSDLRTIDEVHRGERDRVLLQLSGHNRNHRPT
VNNGVVLRHALNQHMAVGANAFLDYEFCKNHLRGSLGGEVIAPQFTLYGNVYAPMSG
WKAAKRAERREERPASGVWDVGVRQLPEALPLAIKGQYFRWSGAADVYFDNGRPQRN
ARGYKYGVYRPVPLVAVGLEQTKVLGGARQTTVQLGVNLSLGEPLSRQLRHQSGPA
FDLQARMGEFVERENRIVLQTRRKHVVLPLTIARVDTDPATGRITVTGVTEPGAQVS
LGLPNNGEVVVAQADGSGTYRATSARDMVGGPVRARATNRHGDRSREVTHHYVDVAVK
GEVPLTLGAVRTHPGTVTGTGKTPGAKVRIDFPDGFVGDVAVNGGDFTVASKG
DVTASGPIVAIARDDDGRESPRRTVQYDDRVNGGSGAPTVVLHTDGTNGRVTVSGK
GRPGDTIRVDFPDGTTKEVVAGPDGYRVTSDRDMTAGDITVSGTDAKGNVGGPVKR
PYHDIFVPVPPTVEVATDSSSGRVTVSGKATPRAVKVDFPGGTSKTVTADADGRYR
ATSDGDVPGGDIVVTQTGMPGAAGKPVRRPYVDTVAPTPMKVTDISMRTDGNSGVVT
VTGYTVGGSTVTFTFDGTTAGTTANDRGKYTVTSTADI PAGPIRVSARGPRNQQGS
ATDHYLDWKQTLLGGKIRLLRPVARLLSPGSMTYTEIAKSFDGSSLGIVARFE
PANGAPPQTAALLAAIKLHDPNYRLESNKMFYIYLDTMNSDPYNRVPNGDYPVTLVLE
DKATGAREATTMVLKVTGSTYKAPVVPGANGVLGTGPGPSLGGSLIGGEGGLGS
(SEQ ID NO:2)

FIG. 2A

LTLGAVRTHPGTVTGTGKTPGAKVRIDFPDGFVGDVAVNGGDFTVASKGDVTA
SGPIVAIARDDDGRESPRRTVQYDDRVNGGSGAPTVVLHTDGTNGRVTVSGKGRPG
DTIRVDFPDGTTKEVVAGPDGYRVTSDRDMTAGDITVSGTDAKGNVGGPVKRPYHD
IFVPVPPTVEVATDSSSGRVTVSGKATPRAVKVDFPGGTSKTVTADADGRYRATSD
GDVPGGDIVVTQTGMPGAAGKPVRRPYVDTVAPTPMKVTDISMRTDGNSGVVTVTGY
TVGGSTVTFTFDGTTAGTTANDRGKYTVTSTADI PAGPIRVSARGPRNQQGSATDH
YLDWKQTLLGGKIRLLRPVARLLSPGSMTYTEIAKSFDGSSLGIVARFE PANG
APPQTAALLAAIKLHDPNYRLESNKMFYIYLDTMNSDPYNRVPNGDYPVTLVLEDKAT
GAREATTMVLKVTGSTYKAPVVPGANGVLGTGPGPSLGGSLIGGEGGLGS
(SEQ ID NO:3)

FIG. 2B

LTLGAVRTHPGTVTGTGKTPGAKVRIDFPDGFVGDVAVNGGDFTVASKGDVTA
SGPIVAIARDDDGRESPRRTVQYDDRVNGGSGAPTVVLHTDGTNGRVT
(SEQ ID NO:4)

FIG. 2C

SKGDVTASGPIVAIARDDDGRESPRRTVQYDDRVNGGSGAPTVVLHTDGTNGRVT
SGKGRPGDTIRVDFPDGTTKEVVAGPDGYRVTSDRDMTAGDI (SEQ ID NO:5)

FIG. 2D

TNGRVTVSGKGRPGDTIRVDFFPDGTTKEVVAGPDGTYRVTSRDRMTAGDITVSGTDA
KGNVGGPVKRPyHDI FVPVPPTVEVATDSSGRVTVSGKATPR (SEQ ID NO:6)

FIG. 2E

TVSGTDAKGNVGGPVKRPyHDI FVPVPPTVEVATDSSGRVTVSGKATPRAKVKVDF
PGGTsKTVTADADGRYRATSDGDVPGGDIVVTQTGMPGAAGKP (SEQ ID NO:7)

FIG. 2F

AKVKVDFPGGTsKTVTADADGRYRATSDGDVPGGDIVVTQTGMPGAAGKPVRPyVD
TVAPTPMKVTIDSMRTDGNSGVVTVTGYTVGGSTVTFTPDGT (SEQ ID NO:8)

FIG. 2G

VRRPyVDTVAPTPMKVTIDSMRTDGNSGVVTVTGYTVGGSTVTFTPDGTAGTTAN
DRGKYTVTSTADIPAGPIRVSARGPRNQQGSATDHYLDATKQ (SEQ ID NO:9)

FIG. 2H

TAGTTANDRGKYTVTSTADIPAGPIRVSARGPRNQQGSATDHYLDATKQTLGGKI
RLLRPVARLLLSPGSMTYTEIAKSFDGSSLGIVARFEPANGAPPQTAAL
(SEQ ID NO:10)

FIG. 2I

TLLGGKIRLLRPVARLLLSPGSMTYTEIAKSFDGSSLGIVARFEPANGAPPQTAAL
LAAIKLHDPNYRLESNKMFIYLDTMNSDPYNRVPNGDYPVTLV
(SEQ ID NO:11)

FIG. 2J

PPQTAALLAAIKLHDPNYRLESNKMFIYLDTMNSDPYNRVPNGDYPVTLVLEDKATG
AREATTMVLKVTGSTYKGAPVVPGANGVLGTGPGPSLGGSLI
(SEQ ID NO:12)

FIG. 2K

LEDKATGAREATTMVLKVTGSTYKGAPVVPGANGVLGTGPGPSLGGSLI
GS (SEQ ID NO:13)

FIG. 2L

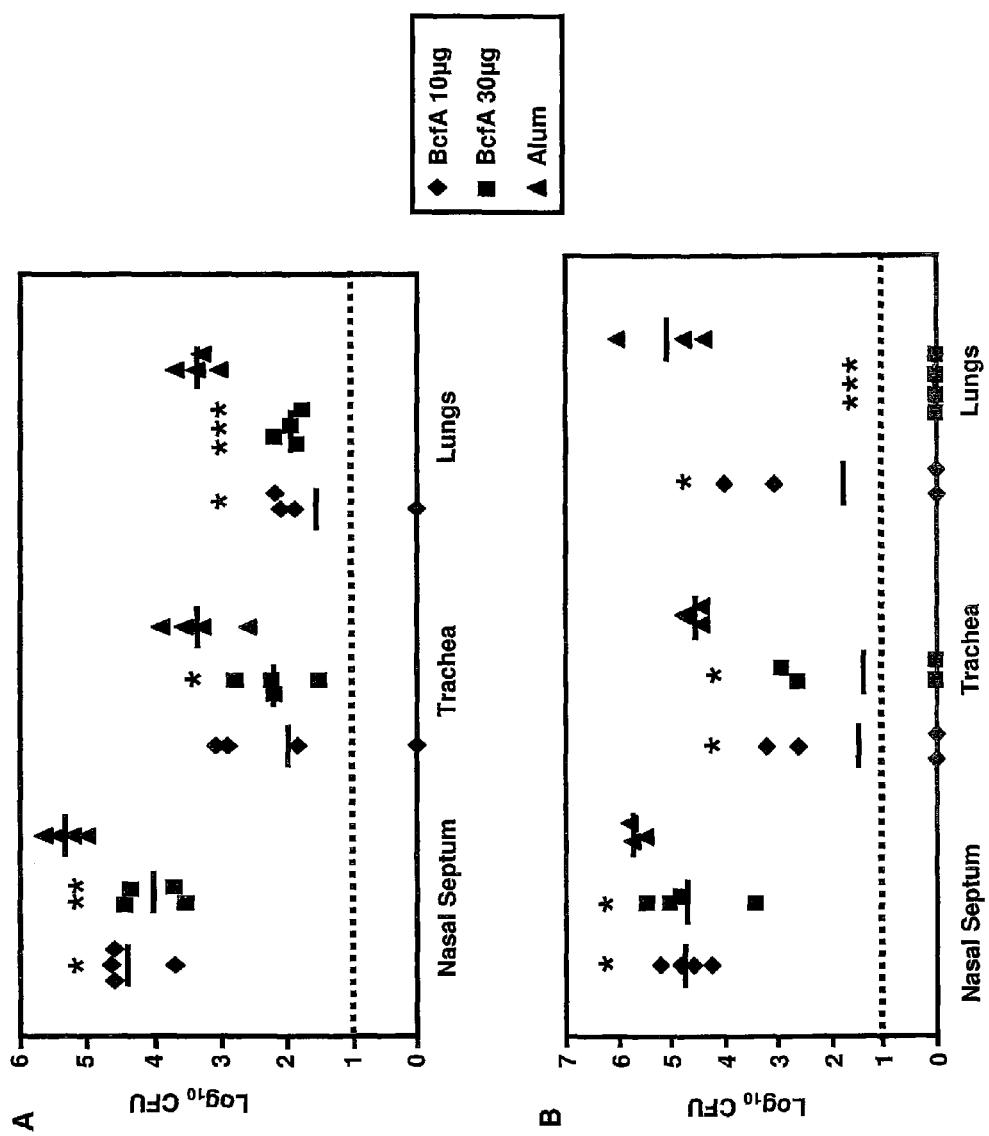


FIG. 3

1

**BORDETELLA OUTER-MEMBRANE
PROTEIN ANTIGENS AND METHODS OF
MAKING AND USING THE SAME**

RELATED APPLICATIONS

This application is a continuation of and claims priority to U.S. patent application Ser. No. 14/498,537, filed Sep. 26, 2014, which is a continuation of and claims priority to U.S. patent application Ser. No. 12/680,823, filed Jul. 16, 2010, now issued as U.S. Pat. No. 8,877,201, which is a 35 U.S.C. §371 national phase application of PCT Application PCT/US2008/012051, filed Oct. 23, 2008, and published in English on Jul. 30, 2009, as International Publication No. WO 2009/094006, and which claims the benefit of U.S. Provisional Patent Application Ser. No. 60/982,513, filed Oct. 25, 2007, the disclosure of each of which is incorporated herein by reference in its entirety.

GOVERNMENT SUPPORT

This invention was made with Government Support under Grant No. NCR-2005-05000 from the USDA. The United States Government has certain rights to this invention.

**STATEMENT REGARDING ELECTRONIC
FILING OF A SEQUENCE LISTING**

A Sequence Listing in ASCII text format, submitted under 37 C.F.R. §1.821, entitled 9151-102_ST25.txt, 38172 bytes in size, generated on Jul. 15, 2010, and filed via EFS-Web, is provided in lieu of a paper copy. This Sequence Listing is hereby incorporated by reference into the specification for its disclosures.

FIELD OF THE INVENTION

The present invention concerns antigens, formulations thereof, and methods of using the same.

BACKGROUND OF THE INVENTION

Bordetellae are Gram-negative bacteria that colonize the respiratory tracts of humans and animals. *Bordetella bronchiseptica* and *Bordetella pertussis* are well-adapted pathogens of the human and animal respiratory tract, respectively. *Bordetella pertussis* infects only humans and causes the acute respiratory disease known as whooping cough. It is estimated that 20-30% of adolescents and adults who have chronic cough lasting for more than one week are infected with *B. pertussis*. The current acellular vaccines, although effective against severe symptoms, are not particularly effective in preventing the carrier state. Adult and adolescent carriers harboring *B. pertussis* in the nasopharynx are responsible for the familial transmission of the bacteria to infants and young children, in whom the disease is severe and sometimes lethal. Thus, the continued presence of *B. pertussis* and *B. parapertussis* and the resurgence of pertussis despite widespread vaccinations, make the development of efficacious vaccines a top priority.

B. bronchiseptica has a broad host range infecting a variety of animals. It typically establishes asymptomatic infections but can cause atrophic rhinitis in pigs, kennel cough in dogs, snuffles in rabbits and bronchopneumonia in guinea pigs. Animals continue to be carriers of *B. bronchiseptica* despite vaccinations and frequently shed bacteria resulting in outbreaks among herds. Since *B. bronchiseptica*

2

can cause respiratory disease in immunocompromized patients, vaccination of pets and food-producing animals with attenuated *B. bronchiseptica* may pose health risks to these patients through zoonotic transmission. Thus, there is a need to develop acellular vaccines for immunizing animals.

SUMMARY OF THE INVENTION

10 This invention is based upon our identification of a gene, designated by us as BcfA (*Bordetella* colonization factor A) by a bioinformatics approach. We produced and purified BcfA-T7-tagged fusion protein from *E. coli*, and have raised anti-sera against the purified protein in rats. Western-blotting with anti-BcfA antibody indicated that BcfA is localized to the outer membrane and that it is expressed during *Bordetella* infection of rats. By intranasal infection of rats, we have shown that BcfA plays an important role in respiratory colonization of *B. bronchiseptica*. We have also found that BcfA is expressed in recent clinical isolates of *B. pertussis* from human patients. Pilot experiments conducted in the laboratory also provide evidence that anti-serum against BcfA is able to protect mice against subsequent challenge with *B. bronchiseptica*. These data indicate that BcfA is useful as a vaccine and that anti-BcfA serum has a protective effect in animals.

20 A first aspect of the invention is an isolated protein or peptide selected from the group consisting of *Bordetella* colonization factor A (BcfA) protein and antigenic fragments thereof. In some embodiments, the BcfA protein has the sequence of SEQ ID NO:2. In some embodiments, the protein or peptide is an antigenic fragment of BcfA from 20 to 500 amino acids in length. In some embodiments, the 30 protein or peptide is an antigenic fragment of BcfA having the sequence given herein as SEQ ID NO:3 or an antigenic fragment comprising 10 or more contiguous amino acids thereof.

35 A further aspect of the invention is an isolated nucleic acid that encodes a protein or peptide as described herein. The nucleic acid may in some embodiments be operatively associated with a promoter, and in some embodiments may be in a host cell that contains the nucleic acid and expresses the encoded protein or peptide.

40 A further aspect of the invention is a method of producing an immune response in a mammalian subject in need thereof, comprising administering the subject a protein or peptide as described herein in an amount effective to produce an immune response in that subject (e.g., a protective immune response to *Bordetella* infection, such as a *Bordetella bronchiseptica* or *Bordetella pertussis* infection).

45 A further aspect of the present invention is a composition comprising a protein or peptide as described herein in a pharmaceutically acceptable carrier.

50 A further aspect of the invention is an isolated antibody (e.g., a monoclonal antibody or polyclonal antibody) that binds to BcfA protein (e.g., a protein of SEQ ID NO:2). In some embodiments the antibody may be coupled to a solid support or a detectable group.

55 A further aspect of the present invention is a composition comprising an antibody as described herein in a pharmaceutically acceptable carrier.

60 A further aspect of the present invention is a method of treating a mammalian subject for a *Bordetella* infection (e.g., a *Bordetella bronchiseptica* or *Bordetella pertussis* infection), comprising administering the subject an antibody as described herein in a treatment effective amount.

A further aspect of the invention is a method of detecting *Bordetella* (e.g., *Bordetella bronchiseptica* or *Bordetella pertussis*) in a biological sample, comprising: contacting the sample to an antibody as described herein; and then detecting the presence or absence of specific binding of the antibody to the sample, the presence of specific binding to the sample indicating the presence of *Bordetella* in the sample.

A still further aspect of the invention is the use of a protein, peptide, or antibody as described herein for the preparation of a medicament for carrying out a method of treatment as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the nucleotide sequence encoding BcfA protein.

FIG. 2A shows the amino acid sequence of full-length BcfA. FIG. 2B shows the predicted 508 amino acid residue extracellular domain of BcfA. FIGS. 2C-2L show fragments of the extracellular domain of BcfA.

FIG. 3 shows that Immunization with BcfA protects mice against *B. bronchiseptica* challenge.

DETAILED DESCRIPTION OF THE INVENTION

Subjects to be treated by the methods of the present invention are generally mammalian subjects, including but not limited to human, monkey, chimpanzee, ape, dog, cat, pig, rabbit, goat, cow, cattle, horse, etc. Subjects may be male or female and may be any age including neonate, infant, juvenile, adolescent, adult, and geriatric subjects.

"Antibodies" as used herein refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE. The antibodies may be monoclonal or polyclonal and may be of any species of origin, including (for example) mouse, rat, rabbit, horse, or human, or may be chimeric antibodies. See, e.g., M. Walker et al., *Molec. Immunol.* 26, 403-11 (1989). The antibodies may be recombinant monoclonal antibodies, for example produced according to the methods disclosed in Reading U.S. Pat. No. 4,474,893, or Cabilly et al., U.S. Pat. No. 4,816,567. The antibodies may be humanized or chimeric antibodies. The antibodies may also be chemically constructed according to methods such as disclosed in Segal et al., U.S. Pat. No. 4,676,980.

"Antigenic fragment" of a protein (e.g., BcfA) as used herein is any portion of the protein that, when administered in accordance with the methods described herein, elicits, in a subject, an immune response that is either a fragment-specific or specific for the protein from which the fragment was obtained. The immune response can be either a humoral or a cell-mediated response. Antigenic fragments are known. See, e.g., U.S. Pat. No. 7,101,987; see also U.S. Pat. Nos. 7,270,816; 7,211,411; 7,163,685; and 7,151,082. Antigenic fragments can be of any suitable length (e.g., from 10, 12 or 20 contiguous amino acids up to 50, 100 or 200 contiguous amino acids or more) and generated by known techniques such as epitope mapping. (e.g., a fragment that includes an epitope region as described below).

1. Antigens

The present invention includes *B. bronchiseptica* compositions composed of one or more *B. bronchiseptica* antigens against which it is desired to generate an immune response. The use of bacterial antigens in the production of antigen compositions and vaccines is well-known in the art and described in, for example, U.S. Pat. No. 7,255,867.

Compositions of the invention may be composed of BcfA (SEQ ID NO:2), the extracellular domain of BcfA (SEQ ID NO:3), or fragments or epitopes thereof. The instant vaccine can be a monovalent vaccine or multi-valent vaccine. Multi-valent vaccines generally include more than one type of antigen and can be produced by mixing a number of different antigens.

The instant antigen(s) can be made using any conventional synthetic or recombinant means. The amino acid sequence of an antigen for use in the invention can be modified to include non-naturally occurring amino acids or to increase the stability of the compound. When the antigen is produced by synthetic means, such amino acids may be introduced during production. The antigen may also be modified following either synthetic or recombinant production.

The antigen for use in the invention may also be produced using D-amino acids. In such cases, the amino acids will be linked in reverse sequence in the C to N orientation. This is conventional in the art for producing such peptides. A number of side chain modifications are also known in the art and may be made to the side chains of the antigen for use in the present invention. Such modifications include, for example, modifications of amino acids by reductive alkylation by reaction with an aldehyde followed by reduction with NABH_4 , amidination with methylacetimidate or acylation with acetic anhydride.

An antigen for use in the invention can be produced in large scale following purification by high pressure liquid chromatography (HPLC) or other techniques after recombinant expression as described herein.

Polynucleotides to produce an antigen for use in the invention can include DNA or RNA. They may also be polynucleotides which include within them synthetic or modified nucleotides. A number of different types of modifications to polynucleotides are known in the art. These include methylphosphate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. Although the techniques mentioned herein are generally well-known in the art, reference may be made in particular to Sambrook and Russell (2001) *Molecular Cloning: A Laboratory Manual*, 3rd Edition, CSHL Press.

An antigen for use in the present invention can be produced by recombinant means by providing a polynucleotide encoding the antigen and, where appropriate, encoding any desired flanking sequences under the control of a promoter and other required sequences. Such a polynucleotide is generally provided in the form of an expression vector.

Such vectors can be transformed into a suitable host cell to provide for expression of an antigen of the invention. Thus, an antigen for use according to the invention can be obtained by cultivating a host cell transformed or transfected with an expression vector as described above under conditions to provide for expression of the antigen, and recovering the expressed antigen.

The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid. Promoters and other expression regulation signals may be selected to be compatible with the host cell for which the expression vector is designed.

Host cells transformed (or transfected) with the polynucleotides or vectors for the replication and expression of

polynucleotides of the invention will be chosen to be compatible with the said vector and preferably will be bacterial, e.g., *E. coli*. Alternatively they may be cells of a human or animal cell line such as CHO or COS cells, or yeast or insect cells. The cells may also be cells of a non-human animal such as a sheep or rabbit or plant cells.

2. Antigen Compositions

An antigen composition of the present invention can also include one or more adjuvants. Adjuvants for use in the production of antigenic compositions such as vaccines are well-known and routinely employed by the skilled artisan. See, e.g., U.S. Pat. No. 7,183,402. For example, adjuvants for parenteral administration include aluminum compounds, such as aluminum hydroxide, aluminum phosphate, and aluminum hydroxy phosphate. The antigen is precipitated with, or adsorbed onto, the aluminum compound according to standard protocols. Other adjuvants, such as RIBI (ImmunoChem, Hamilton, Mont.), are used in parenteral administration.

Adjuvants for mucosal administration include bacterial toxins, e.g., the cholera toxin (CT), the *E. coli* heat-labile toxin (LT), the *Clostridium difficile* toxin A and the pertussis toxin (PT), or combinations, subunits, toxoids, or mutants thereof such as a purified preparation of native cholera toxin subunit B (CTB). Fragments, homologs, derivatives, and fusions to any of these toxins are also suitable, provided that they retain adjuvant activity. Preferably, a mutant having reduced toxicity is used. Suitable mutants are described, e.g., in WO 95/17211, WO 96/06627, and WO 95/34323. Other adjuvants, such as a bacterial monophosphoryl lipid A (MPLA) of, e.g., *E. coli*, *Salmonella minnesota*, *Salmonella typhimurium*, or *Shigella flexneri*; saponins, or polylactide glycolide (PLGA) microspheres, are also be used in mucosal administration.

Adjuvants useful for both mucosal and parenteral administrations include polyphosphazene (WO 95/02415), DC-chol (3 b-(N'-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol (U.S. Pat. No. 5,283,185 and WO 96/14831) and QS-21 (WO 88/09336).

The compositions of the present invention may be administered by any suitable route. The compositions can be formulated for delivery by a mucosal, parenteral or transdermal route. Mucosal delivery routes include nasal, oral and oropharyngeal routes, whereas parenteral routes include intramuscular, intraperitoneal, or subcutaneous injection.

Suitable binders and carriers may also be introduced into the present composition depending on the type of formulation that is provided. Oral formulations typically may include excipients such as, for example, pharmaceutical grades of mannitol, lactose, starch, sodium saccharine, cellulose, and magnesium carbonate. In some embodiments, vaccination is carried out by intranasal delivery of a liquid or spray.

The compositions are administrated in a manner compatible with the dosage formulation in such an amount as will be prophylactically effective. The quantity to be administered depends on a number of factors. These include the subject to be treated, capacity of the subject's immune system to synthesize antibodies and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend on the judgment of the practitioner. In general, the dose per subject may be 5 µg, 50 µg, or 250 µg, up to 10 mg or 100 mg, per dose.

The compositions may be given in a single dose schedule or preferably in a multiple-dose schedule. A multiple-dose schedule is one in which a primary course of vaccination may be with 1 or 2 up to 5 or 10 separate doses, followed by other doses given at subsequent time intervals required to maintain and/or reinforce the immune response, for

example, at 1 to 4 months for a second dose and if needed, a subsequent dose(s) after several months.

3. Antibodies

Polyclonal antibodies used to carry out the present invention may be produced by immunizing a suitable animal (e.g., rabbit, goat, etc.) with an antigen to which a monoclonal antibody to BcfA binds, collecting immune serum from the animal, and separating the polyclonal antibodies from the immune serum, in accordance with known procedures.

10 Monoclonal antibodies used to carry out the present invention may be produced in a hybridoma cell line according to the technique of Kohler and Milstein, *Nature* 265, 495-97 (1975). For example, a solution containing the appropriate antigen may be injected into a mouse and, after 15 a sufficient time, the mouse sacrificed and spleen cells obtained. The spleen cells are then immortalized by fusing them with myeloma cells or with lymphoma cells, typically in the presence of polyethylene glycol, to produce hybridoma cells. The hybridoma cells are then grown in a 20 suitable media and the supernatant screened for monoclonal antibodies having the desired specificity. Monoclonal Fab fragments may be produced in *Escherichia coli* by recombinant techniques known to those skilled in the art. See, e.g., W. Huse, *Science* 246, 1275-81 (1989).

25 Antibodies specific to BcfA can also be obtained by phage display techniques known in the art.

Those skilled in the art will be familiar with numerous specific immunoassay formats and variations thereof which may be useful for carrying out the method disclosed herein.

30 See generally E. Maggio, Enzyme-Immunoassay, (1980) (CRC Press, Inc., Boca Raton, Fla.); see also U.S. Pat. No. 4,727,022 to Skold et al. titled "Methods for Modulating Ligand-Receptor Interactions and their Application," U.S. Pat. No. 4,659,678 to Forrest et al. titled "Immunoassay of Antigens," U.S. Pat. No. 4,376,110 to David et al., titled "Immunometric Assays Using Monoclonal Antibodies," U.S. Pat. No. 4,275,149 to Litman et al., titled "Macromolecular Environment Control in Specific Receptor Assays," U.S. Pat. No. 4,233,402 to Maggio et al., titled "Reagents and Method Employing Channeling," and U.S. Pat. No. 4,230,767 to Boguslaski et al., titled "Heterogenous Specific Binding Assay Employing a Coenzyme as Label." Applicants specifically intend that the disclosures of all U.S. Patent references cited herein be incorporated herein by reference in their entirety.

45 Antibodies as described herein may be conjugated to a solid support suitable for a diagnostic assay (e.g., beads, plates, slides or wells formed from materials such as latex or polystyrene) in accordance with known techniques, such as precipitation. Antibodies as described herein may likewise be conjugated to detectable groups such as radiolabels (e.g., ³⁵S, ¹²⁵I, ¹³¹I), enzyme labels (e.g., horseradish peroxidase, alkaline phosphatase), and fluorescent labels (e.g., fluorescein) in accordance with known techniques. The term "antigenic equivalents" as used herein, refers to proteins or peptides which bind to an antibody which binds to the protein or peptide with which equivalency is sought to be established. Antibodies which are used to select such antigenic equivalents are referred to as "selection antibodies" herein.

4. Utility

60 Antigens of the present invention (BcfA and fragments thereof) and formulations of such antigens are useful for producing an immune response against said antigen in a mammalian subject. Such an immune response is useful for the production of antibodies, which antibodies can be used for diagnostic purposes (in detecting the presence of *Bordetella*) or for therapeutic purposes in treating *Bordetella* by passive immunity as described herein.

Antigens of the present invention are also useful as vaccines for providing protective immunity in mammalian subjects against *Bordetella* infection.

Example 1

Passive Immunization

Groups of five C57/BL6 mice were separately injected intraperitoneally with 200 µl of sera harvested from wild-type inoculated rats, BcfA-specific polyclonal serum, pre-immune sera or sterile phosphate-buffered saline (PBS). Three to four hours after inoculation, these mice were intranasally challenged with 5×10^5 colony forming units (cfus) of wild-type *B. bronchiseptica* strain RB50 in a 25 µl droplet. Seven days post-inoculation, mice were sacrificed and trachea, nasal septum and lungs were harvested in sterile PBS and homogenized. Colonization of these organs was quantified by plating different dilutions of the homogenate in BG blood plates containing 50 µg/ml of streptomycin and subsequent colony counting. The results of this analysis indicated that anti-serum raised against BcfA was able to protect mice against subsequent challenge with *B. bronchiseptica*.

Example 2

BcfA Epitopes

The purified BcfA protein migrates in an SDS-polyacrylamide gel at a mobility corresponding to ≈ 100 kDa, which is consistent with the annotated length (969 amino acids; FIG. 2A) of the BcfA open reading frame. BcfA displays homology to other bacterial proteins including BipA from *Bordetella*, invasins from *Yersinia*, and intimins from enteropathogenic *E. coli*. Based on the known structure of these proteins, the C-terminal 508 amino acid residues of BcfA are expected to encompass the extracellular region of BcfA and thus will interact with the immune system. Accordingly, amino acid residues 461-969 of BcfA (SEQ ID NO:3; FIG. 2B), or one or more fragments thereof, are expected to elicit an immune response against *B. bronchiseptica*. Exemplary fragments of BcfA are shown in FIGS. 2C-2L.

Additional fragments of BcfA include antigenic regions of the BcfA extracellular domain as well as fragments expected to bind to major histocompatibility complex (MHC) class I and MHC class II molecules. Accordingly, the amino acid sequence of the extracellular region of BcfA was analyzed using two independent web-based algorithms that predict antigenic sites in proteins (Table 1) and potential binding to MHC class I and MHC class II molecules (Table 2).

Multiple peptides within the extracellular region of BcfA were predicted to be antigenic and exhibit high binding affinity for human HLA molecules (Table 3).

TABLE 1

Predicted Epitope	SEQ ID NO:	Location	Antigenic Score ¹
GDYPVTLVLED	14	443	1.200
GGPVKRPyHDIFVPVPPTVEVATD	15	165	1.179
APTVVLHT	16	91	1.165

TABLE 1-continued

5	Predicted Epitope	SEQ ID NO:	Location	Antigenic Score ¹
	QTLLGGKIRLLRPVARLLLSP	17	350	1.162
	SGVVTVTGY	18	277	1.143
10	PQTAALLAAIKLHDPN	19	402	1.137
	GKAPVVPGANGV	20	474	1.128
	GKPVRPPYVDTVAPTPMKVTID	21	248	1.119
	GTGVVTVT	22	11	1.110
15	ASGPIVAIA	23	57	1.108
	TMVLKVGTGS	24	463	1.106
	GGSLLIG	25	495	1.095
20	VGGSTTVTVTFP	26	287	1.093
	RAKVKVDFFP	27	200	1.092
	GGDIVVVTQ	28	233	1.089
25	GAVRTH	29	4	1.086
	LDGIVARF	30	387	1.086
	GDVVAG	31	37	1.081
30	SGRVTVSGK	32	188	1.079
	KEVVAGP	33	127	1.078
	RTVQYD	34	76	1.078
35	FTVASKGDV	35	47	1.073
	PAGPIRV SAR	36	321	1.068
	DHYLDA	37	341	1.052
40	GAKVRID	38	24	1.051
	YTVTST	39	312	1.050
	DITVSGT	40	149	1.037

45 Location is the position of the first residue. ¹Score obtained using the Antigenic program which employs the method of Kolaskar and Tongaonkar (1990). FEBS Letters 276: 172-174.

TABLE 2

50	Predicted Epitope	SEQ ID NO:	Location	HLA Molecule	BIMAS Score ^{1,2}
	RRTVQYDDR	41	75	HLA-B_2705	3000
55	LRPVARLLL	42	360	HLA-B_2705	2000
	AREATTMV L	43	458	HLA-B_2705	2000
	IRLLRPV ARL	44	357	HLA-B_2705	2000
60	AREATTMVLK	45	458	HLA-B_2705	2000
	KRPYHDIFV	46	166	HLA-B_2705	1800
	RRTVQYDDRV	47	75	HLA-B_2705	1800
65	GPVKRPYHDI	48	163	HLA-B_5102	1320
	EVATDSSSGR	49	181	HLA-A68.1	1200

US 9,486,514 B2

9

TABLE 2-continued

Predicted Epitope	SEQ ID NO:	Location	HLA Molecule	BIMAS Score ^{1,2}
RPYHDIFVPV	50	167	HLA-B_5102	1100
IRLLRPVAR	51	357	HLA-B_2705	1000
ARFEPANGA	52	392	HLA-B_2705	1000
IRVSARGPR	53	325	HLA-B_2705	1000
VRIDFPDGTF	54	27	HLA-B_2705	1000
VPVPPTVEV	55	174	HLA-B_5102	660
APVVPGANGV	56	476	HLA-B_5102	660
LESNKNMFIYL	57	420	HLA-B60	640
GRPGDTIRV	58	111	HLA-B_2705	600
MRTDGNNSGV	59	271	HLA-B_2705	600
RRPYVDTVA	60	252	HLA-B_2705	600
VRRPYVDTV	61	251	HLA-B_2705	600
YRATSDGDV	62	223	HLA-B_2705	600
VRTHPGTGV	63	6	HLA-B_2705	600
MRTDGNNSGVV	64	271	HLA-B_2705	600
VRTHPGTGVV	65	6	HLA-B_2705	600
NRVPNGDYPV	66	438	HLA-B_2705	600
ARLLLSPGSM	67	364	HLA-B_2705	600
YRLESNKNMFI	68	418	HLA-B_2705	600
FPGGTSKTV	69	207	HLA-B_5102	586
APTPMKVTI	70	260	HLA-B_5101	484
GPSLGGSLLI	71	491	HLA-B_5102	484
GPSLGGSLLI	71	491	HLA-B_5101	440
SPGSMTYTEI	72	369	HLA-B_5101	440
GPVKRPYHDI	48	163	HLA-B_5101	440
APTPMKVTI	70	260	HLA-B_5102	440
SPGSMTYTEI	72	369	HLA-B_5102	440
VVAGPDGTYR	73	129	HLA-A68.1	400
FPGGTSKTV	69	207	HLA-B_5101	381
FPDGTTKEVV	74	121	HLA-B_5101	381
RESPRRTVQY	75	71	HLA-B_4403	360
FPDGTTKEV	76	121	HLA-B_5101	346
AALLAAIKL	77	405	HLA-B_5102	330
MPGAAGKPV	78	243	HLA-B_5101	315
FPDGTFGDV	79	31	HLA-B_5101	315
VAPTPMKVTI	80	259	HLA-B_5101	315
KLHDPNYRL	81	412	HLA-A_0201	307
DAWTQTL	82	345	HLA-B_5102	303

10

TABLE 2-continued

Predicted Epitope	SEQ ID NO:	Location	HLA Molecule	BIMAS Score ^{1,2}
DTMNSDPYNR	83	430	HLA-A68.1	300
YRLESNKNMF	84	418	HLA-B_2705	300
GRVTVSGKGR	85	103	HLA-B_2705	300

Location is the position of the first residue. ¹Score obtained using the BIMAS program developed by Parker, et al. (1994) J. Immunol. 152: 163, which provides the rank potential of 8-mer, 9-mer, or 10-mer peptides based on a predicted half-time of dissociation to HLA class I molecules.

²Minimum scores 300 on the BIMAS site were used.

TABLE 3

Predicted Epitope	SEQ ID NO:	Loca- tion	Antigenic Score	BIMAS Score
RTVQYD	34	76	1.179	
RRTVQYDDR	41	75		
RRTVQYDDRV	47	75		1800
GGPVKRPYHDIFVPVPPTVEVATD	15	165	1.179	
KRPYHDIFV	46	166		1800
RPYHDIFVPV	50	167		
VPVPPTVEV	55	174		
GKPVRPPYVDTVAPTPMKVTID	21	248	1.119	
VRRPYVDTV	61	251		
RRPYVDTVA	60	252		
VAPTPMKVTI	80	259		
APTPMKVTI	70	260		315
QTLLGGKIRLLRPVARLLSP	17	350	1.162	
IRLLRPVARL	44	357		
IRLLRPVAR	51	357		
LRPVARLLL	42	360		2000
PQTAALLAAIKLHDPN	19	402	1.137	
AALLAAIKL	77	405		330
GKAPVVPGANGV	20	474	1.128	
APVVPGANGV	56	476		660

Example 3

Active Immunization

FIG. 3 shows that Immunization with BcfA protects mice against *B. bronchiseptica* challenge. Mice were immunized intraperitoneally at 0 and 3 weeks with either 10 or 30 µg of BcfA adsorbed to alum or alum only. One week after the second immunization, mice were intranasally challenged with 5×10^5 CFU or RB50 in a 25 µl volume. Mice were sacrificed at 1 day (FIG. 3A) and 6 days (FIG. 3B) post-

11

challenge and the number of CFU was determined in the nasal septum, trachea and lungs. Individual symbols represent a single mouse. The dashed line represents the lower limits of CFU detection. Black bars represent mean colonization of respective groups. A statistical analysis was carried out using an unpaired two-tailed Student t test. The asterisks

12

indicate the range of the different P values (one asterisk, ≤ 0.05 ; two asterisks, ≤ 0.005 and three asterisks, ≤ 0.0005).

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

SEQUENCE LISTING

```

<160> NUMBER OF SEQ ID NOS: 85

<210> SEQ ID NO 1
<211> LENGTH: 2910
<212> TYPE: DNA
<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 1

gtgaagcaag ccatccacgc cgttgcgttc cgccatgatg cgctcgacgc agtccccgt 60
gtccatcgcc gcccggcgc cggcgcgatg gctggcgct tgacgctgca aaccgtggcg 120
ccggcatttg cccagggggc gccgttttc tccgccccgc ccgcgcaggc cgatcgccag 180
gatgccgcgc acagcgcgat gctgcgggtc ggcgcagacgg cgcccaatt ggcgcacagg 240
caggctgcgc gttcgcgcgc ctgcggcgcc gtggacggcg acttgctgaa aggacaggcc 300
gaggcgccagg ccaatgaggc gtcgcaggaa ggggtggcc tggccaacca gactgaattg 360
ccgttccgtc gccgggttgcg aggccccgtt aattatgact ttgcgaacaa ggacctgtcg 420
ttggatcttc gtaccatcga cgaagtgcattt cgcggcgacgc ggcaccgcgt cttgctgcaa 480
ctgagcggcc acaatcgcaaa tcatacgcccc accgtcaacgc gtggcgctgg tttgcgcatt 540
gccttgaacc agcacatggc cgtggggccg aacgcatttc ttgattacga gttcgccaaag 600
aaccatctgc ggggctcgat gggcgccggag gtcattgcgc cgcaggatcac gctgtatggc 660
aacgtctacg cggccatgtc gggatggaaa gggcccaagc gggccggcgcc cgcgcacagg 720
cggcccgccg cgggctgggg cgttggcgctg cgcctgcac ccggaggcgct gcctggccctg 780
gcaatcaagg gccagtttcc cgcgtggaggc ggcgcggcccg tggattactt cgacaacggc 840
cgtccgcgcg gcaatgcgcg cggctataag tacggcggtt agtaccggcc cgtgcgttg 900
gtggcggtgg gccttggaaaca gaccaagggtt ctcggcgccg cgcgcacagac cactgtgcag 960
cttggcgctca atctcagecc gggcgagccc ttgtccaggc agttgcggca ccagtccggg 1020
ccggcggttcg acttgcaggc cgcgcattggc gaattcgatc agcgtgaaaa ccgcattcg 1080
cttcagacgcg gccgcacggc cgttgcgttg cgcgtgcacgc tcgcgcgcgt cgataccgt 1140
ccggcaacccg ggcggatcac ggttacccggc gtcacccggc cggggccgc ggtcagccctg 1200
gggctgcccc atggcgaagt cgtggcgccg caggccatgcg gcacggaaac ctaccggcg 1260
acgtcgccgc ggcacatggt gggcgcccg gtgcgggctc ggcacacgaa ccgtcatggc 1320
gaccgttagcc gggaaagtccac gcaccattac gtggatcg cggtaacggg cgaggatccg 1380
ctgaegctcg ggcgtgtcg cgcgcattcc ggcacccggc tcgtgaccgt gacccggcaag 1440
accggccctg ggcgcacagggt ggcgcacatgg tttcccgacgc gtcacgtcg tgatgtggc 1500
ccggcaatg gggcgattt cgcgtgcgc tcgaaaggcg atgtgacggc cgcggcccg 1560
atcggtggcgat ttggccgcgc tgacgacggg cggggaaagcc cccgcgtac tgcgtggcg 1620
gacgacaggc tcaatggcg tggctcgccg ggcgcacgg tggtgctgca taccggcg 1680
accaacggcgc ggcgtgcacggt cagcggcaaa ggacggcccg ggcacacgt cagggtggac 1740
ttcccccgaacg gcaccaccaa ggaggtgggtg gggggcccg acggcaccta ccgcgtcaccg 1800
tccgaccggc acatgacggc gggcgacata acgggtgtccg gtaccgatgc caaggcaac 1860

```

US 9,486,514 B2

13

14

-continued

gtgggtggtc ctgtcaagcg tccctaccac gacatcttcg tgcccgtgcc gcccaccgtg 1920
 gaggtggcga ccgactcgta cageggccgc gtcacggta gggcaaggc cacggccgc 1980
 gccaaggta aggtcgattt cccggggggg acgtccaaga ccgtcaccgc cgacgcccac 2040
 ggccgtatac gcgcgaccc tcgatggcgc acgtccatcg tgcaccccg 2100
 accgggatgc cggggcgtgc gggcaagcgg gtgcgtcgac cgtatgtcga tacggtgcc 2160
 ccgacgcgcgta tgaaagtgc catcgacacgc atgcgcacgg acggcaacag cggcgtcg 2220
 acgggtacgg gctacacggt cggcggctcc acgggtacgg tgacccccc cgacggcacg 2280
 accggccgta caaccgcggaa tgaccggaggc aaatacacgg taacgtcgac cgccgacatt 2340
 cctgccccgtc cgatccgcgt cagcgcgcgc ggaccgcgc accagcaggc cagcgcgc 2400
 gaccattacc tcgatgcgtg gaccaagcgg acgctgtcg gggcaagat tcgccttc 2460
 cggccgggtcg cgaggctgtt gctgagcccg ggcagcatga catataccga aatgcacaag 2520
 tcgttcgatg gcagttcgct cgacggcatc gtggcacggt tcgagccggc aaacggagca 2580
 cccgcccaga cggccggcgct gctggccggcg atcaagctgc acgateccaa ttatccgt 2640
 gagtccaaca agatgttcat ctatctcgac accatgaaca gcgacccgta caaccgtgtt 2700
 cccaaacggcg attatccgt cacgctgggtt ctcgaggaca aggccaccgg ggcgcgggag 2760
 ggcgaccacca tggtcctgaa ggtgaccggc agtacctatg gcaaagcccc ggtcgtcccc 2820
 ggccgcgaatg gtgtgcttgg cacggggccc ggcccgctgt tggccggcag tctgctgatc 2880
 ggtggcgagg gcccgcgtgtt gggaaagctga 2910

<210> SEQ_ID NO 2

<211> LENGTH: 969

<212> TYPE: PRT

<213> ORGANISM: *Bordetella bronchiseptica*

<400> SEQUENCE: 2

Met	Lys	Gln	Ala	Ile	His	Ala	Val	Ala	Phe	Arg	His	Asp	Ala	Leu	Ala	
1							5							10		15

Arg	Val	Gly	Arg	Val	His	Arg	Arg	Gly	Ala	Ala	Ala	Leu	Ala	Gly	
							20						25		30

Val	Leu	Thr	Leu	Gln	Thr	Val	Ala	Pro	Ala	Phe	Ala	Gln	Gly	Ala	Pro
							35						40		45

Ser	Phe	Ser	Ala	Arg	Pro	Ala	Gln	Ala	Asp	Arg	Gln	Asp	Ala	Ala	Asp
							50						55		60

Ser	Ala	Met	Leu	Arg	Val	Ala	Gln	Thr	Ala	Arg	Gln	Leu	Ala	Gln	Arg
							65						70		80

Gln	Ala	Ala	Gly	Ser	Arg	Ala	Ser	Ala	Arg	Val	Asp	Gly	Asp	Leu	Leu
							85						90		95

Lys	Gly	Gln	Ala	Glu	Ala	Gln	Ala	Asn	Glu	Leu	Leu	Gln	Gly	Val	
							100						105		110

Arg	Leu	Ala	Asn	Gln	Thr	Glu	Leu	Pro	Phe	Leu	Arg	Arg	Leu	Gln	Gly
							115						120		125

Gly	Val	Asn	Tyr	Asp	Phe	Ser	Asn	Lys	Asp	Leu	Ser	Leu	Asp	Leu	Arg
							130						135		140

Thr	Ile	Asp	Glu	Val	His	Arg	Gly	Glu	Arg	Asp	Arg	Val	Leu	Leu	Gln
							145						150		160

Leu	Ser	Gly	His	Asn	Arg	Asn	His	Arg	Pro	Thr	Val	Asn	Gly	Gly	Val
							165						170		175

Val	Leu	Arg	His	Ala	Leu	Asn	Gln	Met	Ala	Val	Gly	Ala	Asn	Ala	
							180						185		190

US 9,486,514 B2

15**16**

-continued

Phe Leu Asp Tyr Glu Phe Gly Lys Asn His Leu Arg Gly Ser Leu Gly
195 200 205

Gly Glu Val Ile Ala Pro Gln Phe Thr Leu Tyr Gly Asn Val Tyr Ala
210 215 220

Pro Met Ser Gly Trp Lys Ala Ala Lys Arg Ala Glu Arg Arg Glu Glu
225 230 235 240

Arg Pro Ala Ser Gly Trp Asp Val Gly Val Arg Leu Gln Pro Glu Ala
245 250 255

Leu Pro Gly Leu Ala Ile Lys Gly Gln Tyr Phe Arg Trp Ser Gly Ala
260 265 270

Ala Val Asp Tyr Phe Asp Asn Gly Arg Pro Gln Arg Asn Ala Arg Gly
275 280 285

Tyr Lys Tyr Gly Val Glu Tyr Arg Pro Val Pro Leu Val Ala Val Gly
290 295 300

Leu Glu Gln Thr Lys Val Leu Gly Gly Ala Arg Gln Thr Thr Val Gln
305 310 315 320

Leu Gly Val Asn Leu Ser Leu Gly Glu Pro Leu Ser Arg Gln Leu Arg
325 330 335

His Gln Ser Gly Pro Ala Phe Asp Leu Gln Ala Arg Met Gly Glu Phe
340 345 350

Val Glu Arg Glu Asn Arg Ile Val Leu Gln Thr Arg Arg Lys His Val
355 360 365

Val Leu Pro Leu Thr Ile Ala Arg Val Asp Thr Asp Pro Ala Thr Gly
370 375 380

Arg Ile Thr Val Thr Gly Val Thr Glu Pro Gly Ala Gln Val Ser Leu
385 390 395 400

Gly Leu Pro Asn Gly Glu Val Val Val Ala Gln Ala Asp Gly Ser Gly
405 410 415

Thr Tyr Arg Ala Thr Ser Ala Arg Asp Met Val Gly Gly Pro Val Arg
420 425 430

Ala Arg Ala Thr Asn Arg His Gly Asp Arg Ser Arg Glu Val Thr His
435 440 445

His Tyr Val Asp Val Ala Val Lys Gly Glu Val Pro Leu Thr Leu Gly
450 455 460

Ala Val Arg Thr His Pro Gly Thr Gly Val Val Thr Val Thr Gly Lys
465 470 475 480

Thr Gly Pro Gly Ala Lys Val Arg Ile Asp Phe Pro Asp Gly Thr Phe
485 490 495

Gly Asp Val Val Ala Gly Asn Gly Gly Asp Phe Thr Val Ala Ser Lys
500 505 510

Gly Asp Val Thr Ala Ser Gly Pro Ile Val Ala Ile Ala Arg Asp Asp
515 520 525

Asp Gly Arg Glu Ser Pro Arg Arg Thr Val Gln Tyr Asp Asp Arg Val
530 535 540

Asn Gly Gly Ser Gly Ala Pro Thr Val Val Leu His Thr Asp Gly
545 550 555 560

Thr Asn Gly Arg Val Thr Val Ser Gly Lys Gly Arg Pro Gly Asp Thr
565 570 575

Ile Arg Val Asp Phe Pro Asp Gly Thr Thr Lys Glu Val Val Ala Gly
580 585 590

Pro Asp Gly Thr Tyr Arg Val Thr Ser Asp Arg Asp Met Thr Ala Gly
595 600 605

US 9,486,514 B2

17**18**

-continued

Asp Ile Thr Val Ser Gly Thr Asp Ala Lys Gly Asn Val Gly Gly Pro
 610 615 620
 Val Lys Arg Pro Tyr His Asp Ile Phe Val Pro Val Pro Pro Thr Val
 625 630 635 640
 Glu Val Ala Thr Asp Ser Ser Ser Gly Arg Val Thr Val Ser Gly Lys
 645 650 655
 Ala Thr Pro Arg Ala Lys Val Lys Val Asp Phe Pro Gly Gly Thr Ser
 660 665 670
 Lys Thr Val Thr Ala Asp Ala Asp Gly Arg Tyr Arg Ala Thr Ser Asp
 675 680 685
 Gly Asp Val Pro Gly Gly Asp Ile Val Val Thr Gln Thr Gly Met Pro
 690 695 700
 Gly Ala Ala Gly Lys Pro Val Arg Arg Pro Tyr Val Asp Thr Val Ala
 705 710 715 720
 Pro Thr Pro Met Lys Val Thr Ile Asp Ser Met Arg Thr Asp Gly Asn
 725 730 735
 Ser Gly Val Val Thr Val Thr Gly Tyr Thr Val Gly Gly Ser Thr Val
 740 745 750
 Thr Val Thr Phe Pro Asp Gly Thr Thr Ala Gly Thr Thr Ala Asn Asp
 755 760 765
 Arg Gly Lys Tyr Thr Val Thr Ser Thr Ala Asp Ile Pro Ala Gly Pro
 770 775 780
 Ile Arg Val Ser Ala Arg Gly Pro Arg Asn Gln Gln Gly Ser Ala Thr
 785 790 795 800
 Asp His Tyr Leu Asp Ala Trp Thr Lys Gln Thr Leu Leu Gly Gly Lys
 805 810 815
 Ile Arg Leu Leu Arg Pro Val Ala Arg Leu Leu Leu Ser Pro Gly Ser
 820 825 830
 Met Thr Tyr Thr Glu Ile Ala Lys Ser Phe Asp Gly Ser Ser Leu Asp
 835 840 845
 Gly Ile Val Ala Arg Phe Glu Pro Ala Asn Gly Ala Pro Pro Gln Thr
 850 855 860
 Ala Ala Leu Leu Ala Ala Ile Lys Leu His Asp Pro Asn Tyr Arg Leu
 865 870 875 880
 Glu Ser Asn Lys Met Phe Ile Tyr Leu Asp Thr Met Asn Ser Asp Pro
 885 890 895
 Tyr Asn Arg Val Pro Asn Gly Asp Tyr Pro Val Thr Leu Val Leu Glu
 900 905 910
 Asp Lys Ala Thr Gly Ala Arg Glu Ala Thr Thr Met Val Leu Lys Val
 915 920 925
 Thr Gly Ser Thr Tyr Gly Lys Ala Pro Val Val Pro Gly Ala Asn Gly
 930 935 940
 Val Leu Gly Thr Gly Pro Gly Pro Ser Leu Gly Gly Ser Leu Leu Ile
 945 950 955 960
 Gly Gly Glu Gly Gly Leu Leu Gly Ser
 965

<210> SEQ ID NO 3

<211> LENGTH: 509

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

-continued

<400> SEQUENCE: 3

Leu Thr Leu Gly Ala Val Arg Thr His Pro Gly Thr Gly Val Val Thr
 1 5 10 15

Val Thr Gly Lys Thr Gly Pro Gly Ala Lys Val Arg Ile Asp Phe Pro
 20 25 30

Asp Gly Thr Phe Gly Asp Val Val Ala Gly Asn Gly Gly Asp Phe Thr
 35 40 45

Val Ala Ser Lys Gly Asp Val Thr Ala Ser Gly Pro Ile Val Ala Ile
 50 55 60

Ala Arg Asp Asp Asp Gly Arg Glu Ser Pro Arg Arg Thr Val Gln Tyr
 65 70 75 80

Asp Asp Arg Val Asn Gly Gly Ser Gly Ala Pro Thr Val Val Leu
 85 90 95

His Thr Asp Gly Thr Asn Gly Arg Val Thr Val Ser Gly Lys Gly Arg
 100 105 110

Pro Gly Asp Thr Ile Arg Val Asp Phe Pro Asp Gly Thr Thr Lys Glu
 115 120 125

Val Val Ala Gly Pro Asp Gly Thr Tyr Arg Val Thr Ser Asp Arg Asp
 130 135 140

Met Thr Ala Gly Asp Ile Thr Val Ser Gly Thr Asp Ala Lys Gly Asn
 145 150 155 160

Val Gly Gly Pro Val Lys Arg Pro Tyr His Asp Ile Phe Val Pro Val
 165 170 175

Pro Pro Thr Val Glu Val Ala Thr Asp Ser Ser Ser Gly Arg Val Thr
 180 185 190

Val Ser Gly Lys Ala Thr Pro Arg Ala Lys Val Lys Val Asp Phe Pro
 195 200 205

Gly Gly Thr Ser Lys Thr Val Thr Ala Asp Ala Asp Gly Arg Tyr Arg
 210 215 220

Ala Thr Ser Asp Gly Asp Val Pro Gly Gly Asp Ile Val Val Thr Gln
 225 230 235 240

Thr Gly Met Pro Gly Ala Ala Gly Lys Pro Val Arg Arg Pro Tyr Val
 245 250 255

Asp Thr Val Ala Pro Thr Pro Met Lys Val Thr Ile Asp Ser Met Arg
 260 265 270

Thr Asp Gly Asn Ser Gly Val Val Thr Val Thr Gly Tyr Thr Val Gly
 275 280 285

Gly Ser Thr Val Thr Val Thr Phe Pro Asp Gly Thr Thr Ala Gly Thr
 290 295 300

Thr Ala Asn Asp Arg Gly Lys Tyr Thr Val Thr Ser Thr Ala Asp Ile
 305 310 315 320

Pro Ala Gly Pro Ile Arg Val Ser Ala Arg Gly Pro Arg Asn Gln Gln
 325 330 335

Gly Ser Ala Thr Asp His Tyr Leu Asp Ala Trp Thr Lys Gln Thr Leu
 340 345 350

Leu Gly Gly Lys Ile Arg Leu Leu Arg Pro Val Ala Arg Leu Leu Leu
 355 360 365

Ser Pro Gly Ser Met Thr Tyr Thr Glu Ile Ala Lys Ser Phe Asp Gly
 370 375 380

Ser Ser Leu Asp Gly Ile Val Ala Arg Phe Glu Pro Ala Asn Gly Ala
 385 390 395 400

Pro Pro Gln Thr Ala Ala Leu Leu Ala Ile Lys Leu His Asp Pro
 405 410 415

-continued

Asn Tyr Arg Leu Glu Ser Asn Lys Met Phe Ile Tyr Leu Asp Thr Met
 420 425 430

Asn Ser Asp Pro Tyr Asn Arg Val Pro Asn Gly Asp Tyr Pro Val Thr
 435 440 445

Leu Val Leu Glu Asp Lys Ala Thr Gly Ala Arg Glu Ala Thr Thr Met
 450 455 460

Val Leu Lys Val Thr Gly Ser Thr Tyr Gly Lys Ala Pro Val Val Pro
 465 470 475 480

Gly Ala Asn Gly Val Leu Gly Thr Gly Pro Gly Pro Ser Leu Gly Gly
 485 490 495

Ser Leu Leu Ile Gly Gly Glu Gly Leu Leu Gly Ser
 500 505

<210> SEQ ID NO 4

<211> LENGTH: 100

<212> TYPE: PRT

<213> ORGANISM: *Bordetella bronchiseptica*

<400> SEQUENCE: 4

Leu Thr Leu Gly Ala Val Arg Thr His Pro Gly Thr Gly Val Val Thr
 1 5 10 15

Val Thr Gly Lys Thr Gly Pro Gly Ala Lys Val Arg Ile Asp Phe Pro
 20 25 30

Asp Gly Thr Phe Gly Asp Val Val Ala Gly Asn Gly Gly Asp Phe Thr
 35 40 45

Val Ala Ser Lys Gly Asp Val Thr Ala Ser Gly Pro Ile Val Ala Ile
 50 55 60

Ala Arg Asp Asp Asp Gly Arg Glu Ser Pro Arg Arg Thr Val Gln Tyr
 65 70 75 80

Asp Asp Arg Val Asn Gly Gly Ser Gly Ala Pro Thr Val Val Leu
 85 90 95

His Thr Asp Gly
 100

<210> SEQ ID NO 5

<211> LENGTH: 100

<212> TYPE: PRT

<213> ORGANISM: *Bordetella bronchiseptica*

<400> SEQUENCE: 5

Ser Lys Gly Asp Val Thr Ala Ser Gly Pro Ile Val Ala Ile Ala Arg
 1 5 10 15

Asp Asp Asp Gly Arg Glu Ser Pro Arg Arg Thr Val Gln Tyr Asp Asp
 20 25 30

Arg Val Asn Gly Gly Ser Gly Ala Pro Thr Val Val Leu His Thr
 35 40 45

Asp Gly Thr Asn Gly Arg Val Thr Val Ser Gly Lys Gly Arg Pro Gly
 50 55 60

Asp Thr Ile Arg Val Asp Phe Pro Asp Gly Thr Thr Lys Glu Val Val
 65 70 75 80

Ala Gly Pro Asp Gly Thr Tyr Arg Val Thr Ser Asp Arg Asp Met Thr
 85 90 95

Ala Gly Asp Ile
 100

-continued

<210> SEQ_ID NO 6
<211> LENGTH: 100
<212> TYPE: PRT
<213> ORGANISM: *Bordetella bronchiseptica*

<400> SEQUENCE: 6

Ser	Lys	Gly	Asp	Val	Thr	Ala	Ser	Gly	Pro	Ile	Val	Ala	Ile	Ala	Arg
1				5					10				15		

Asp Asp Asp Gly Arg Glu Ser Pro Arg Arg Thr Val Gln Tyr Asp Asp

20				25					30						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Arg Val Asn Gly Gly Ser Gly Ala Pro Thr Val Val Leu His Thr

35				40					45						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Asp Gly Thr Asn Gly Arg Val Thr Val Ser Gly Lys Gly Arg Pro Gly

50				55					60						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Asp Thr Ile Arg Val Asp Phe Pro Asp Gly Thr Thr Lys Glu Val Val

65				70					75				80		
----	--	--	--	----	--	--	--	--	----	--	--	--	----	--	--

Ala Gly Pro Asp Gly Thr Tyr Arg Val Thr Ser Asp Arg Asp Met Thr

85				90					95						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Ala Gly Asp Ile

100															
-----	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

<210> SEQ_ID NO 7
<211> LENGTH: 100
<212> TYPE: PRT
<213> ORGANISM: *Bordetella bronchiseptica*

<400> SEQUENCE: 7

Ser	Lys	Gly	Asp	Val	Thr	Ala	Ser	Gly	Pro	Ile	Val	Ala	Ile	Ala	Arg
1				5					10				15		

Asp Asp Asp Gly Arg Glu Ser Pro Arg Arg Thr Val Gln Tyr Asp Asp

20				25					30						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Arg Val Asn Gly Gly Ser Gly Ala Pro Thr Val Val Leu His Thr

35				40					45						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Asp Gly Thr Asn Gly Arg Val Thr Val Ser Gly Lys Gly Arg Pro Gly

50				55					60						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Asp Thr Ile Arg Val Asp Phe Pro Asp Gly Thr Thr Lys Glu Val Val

65				70					75				80		
----	--	--	--	----	--	--	--	--	----	--	--	--	----	--	--

Ala Gly Pro Asp Gly Thr Tyr Arg Val Thr Ser Asp Arg Asp Met Thr

85				90					95						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Ala Gly Asp Ile

100															
-----	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

<210> SEQ_ID NO 8
<211> LENGTH: 100
<212> TYPE: PRT
<213> ORGANISM: *Bordetella bronchiseptica*

<400> SEQUENCE: 8

Ala	Lys	Val	Lys	Val	Asp	Phe	Pro	Gly	Thr	Ser	Lys	Thr	Val	Thr	
1					5				10			15			

Ala Asp Ala Asp Gly Arg Tyr Arg Ala Thr Ser Asp Gly Asp Val Pro

20				25					30						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Gly Gly Asp Ile Val Val Thr Gln Thr Gly Met Pro Gly Ala Ala Gly

35				40					45						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Lys Pro Val Arg Arg Pro Tyr Val Asp Thr Val Ala Pro Thr Pro Met

50				55					60						
----	--	--	--	----	--	--	--	--	----	--	--	--	--	--	--

Lys Val Thr Ile Asp Ser Met Arg Thr Asp Gly Asn Ser Gly Val Val

65				70					75				80		
----	--	--	--	----	--	--	--	--	----	--	--	--	----	--	--

-continued

Thr Val Thr Gly Tyr Thr Val Gly Gly Ser Thr Val Thr Val Thr Phe
85 90 95

Pro Asp Gly Thr
100

<210> SEQ ID NO 9
<211> LENGTH: 100
<212> TYPE: PRT
<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 9

Val Arg Arg Pro Tyr Val Asp Thr Val Ala Pro Thr Pro Met Lys Val
1 5 10 15

Thr Ile Asp Ser Met Arg Thr Asp Gly Asn Ser Gly Val Val Thr Val
20 25 30

Thr Gly Tyr Thr Val Gly Gly Ser Thr Val Thr Val Thr Phe Pro Asp
35 40 45

Gly Thr Thr Ala Gly Thr Thr Ala Asn Asp Arg Gly Lys Tyr Thr Val
50 55 60

Thr Ser Thr Ala Asp Ile Pro Ala Gly Pro Ile Arg Val Ser Ala Arg
65 70 75 80

Gly Pro Arg Asn Gln Gln Gly Ser Ala Thr Asp His Tyr Leu Asp Ala
85 90 95

Trp Thr Lys Gln
100

<210> SEQ ID NO 10
<211> LENGTH: 100
<212> TYPE: PRT
<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 10

Thr Ala Gly Thr Thr Ala Asn Asp Arg Gly Lys Tyr Thr Val Thr Ser
1 5 10 15

Thr Ala Asp Ile Pro Ala Gly Pro Ile Arg Val Ser Ala Arg Gly Pro
20 25 30

Arg Asn Gln Gln Gly Ser Ala Thr Asp His Tyr Leu Asp Ala Trp Thr
35 40 45

Lys Gln Thr Leu Leu Gly Gly Lys Ile Arg Leu Leu Arg Pro Val Ala
50 55 60

Arg Leu Leu Leu Ser Pro Gly Ser Met Thr Tyr Thr Glu Ile Ala Lys
65 70 75 80

Ser Phe Asp Gly Ser Ser Leu Asp Gly Ile Val Ala Arg Phe Glu Pro
85 90 95

Ala Asn Gly Ala
100

<210> SEQ ID NO 11
<211> LENGTH: 100
<212> TYPE: PRT
<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 11

Thr Leu Leu Gly Gly Lys Ile Arg Leu Leu Arg Pro Val Ala Arg Leu
1 5 10 15

Leu Leu Ser Pro Gly Ser Met Thr Tyr Thr Glu Ile Ala Lys Ser Phe
20 25 30

-continued

Asp Gly Ser Ser Leu Asp Gly Ile Val Ala Arg Phe Glu Pro Ala Asn
 35 40 45

Gly Ala Pro Pro Gln Thr Ala Ala Leu Leu Ala Ala Ile Lys Leu His
 50 55 60

Asp Pro Asn Tyr Arg Leu Glu Ser Asn Lys Met Phe Ile Tyr Leu Asp
 65 70 75 80

Thr Met Asn Ser Asp Pro Tyr Asn Arg Val Pro Asn Gly Asp Tyr Pro
 85 90 95

Val Thr Leu Val
 100

<210> SEQ ID NO 12
 <211> LENGTH: 100
 <212> TYPE: PRT
 <213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 12

Pro Pro Gln Thr Ala Ala Leu Leu Ala Ala Ile Lys Leu His Asp Pro
 1 5 10 15

Asn Tyr Arg Leu Glu Ser Asn Lys Met Phe Ile Tyr Leu Asp Thr Met
 20 25 30

Asn Ser Asp Pro Tyr Asn Arg Val Pro Asn Gly Asp Tyr Pro Val Thr
 35 40 45

Leu Val Leu Glu Asp Lys Ala Thr Gly Ala Arg Glu Ala Thr Thr Met
 50 55 60

Val Leu Lys Val Thr Gly Ser Thr Tyr Gly Lys Ala Pro Val Val Pro
 65 70 75 80

Gly Ala Asn Gly Val Leu Gly Thr Gly Pro Gly Pro Ser Leu Gly Gly
 85 90 95

Ser Leu Leu Ile
 100

<210> SEQ ID NO 13
 <211> LENGTH: 59
 <212> TYPE: PRT
 <213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 13

Leu Glu Asp Lys Ala Thr Gly Ala Arg Glu Ala Thr Thr Met Val Leu
 1 5 10 15

Lys Val Thr Gly Ser Thr Tyr Gly Lys Ala Pro Val Val Pro Gly Ala
 20 25 30

Asn Gly Val Leu Gly Thr Gly Pro Gly Pro Ser Leu Gly Gly Ser Leu
 35 40 45

Leu Ile Gly Gly Glu Gly Leu Leu Gly Ser
 50 55

<210> SEQ ID NO 14
 <211> LENGTH: 11
 <212> TYPE: PRT
 <213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 14

Gly Asp Tyr Pro Val Thr Leu Val Leu Glu Asp
 1 5 10

-continued

<210> SEQ ID NO 15
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: *Bordetella bronchiseptica*
<400> SEQUENCE: 15

Gly	Gly	Pro	Val	Lys	Arg	Pro	Tyr	His	Asp	Ile	Phe	Val	Pro	Val	Pro
1															15
Pro	Thr	Val	Glu	Val	Ala	Thr	Asp								
															20

<210> SEQ ID NO 16
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: *Bordetella bronchiseptica*
<400> SEQUENCE: 16

Ala	Pro	Thr	Val	Val	Leu	His	Thr
1							5

<210> SEQ ID NO 17
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: *Bordetella bronchiseptica*
<400> SEQUENCE: 17

Gln	Thr	Leu	Leu	Gly	Gly	Lys	Ile	Arg	Leu	Leu	Arg	Pro	Val	Ala	Arg
1															15
Leu	Leu	Leu	Ser	Pro											20

<210> SEQ ID NO 18
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: *Bordetella bronchiseptica*
<400> SEQUENCE: 18

Ser	Gly	Val	Val	Thr	Val	Thr	Gly	Tyr
1								5

<210> SEQ ID NO 19
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: *Bordetella bronchiseptica*
<400> SEQUENCE: 19

Pro	Gln	Thr	Ala	Ala	Leu	Leu	Ala	Ala	Ile	Lys	Leu	His	Asp	Pro	Asn
1															15

<210> SEQ ID NO 20
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: *Bordetella bronchiseptica*
<400> SEQUENCE: 20

Gly	Lys	Ala	Pro	Val	Val	Pro	Gly	Ala	Asn	Gly	Val	
1											10	

<210> SEQ ID NO 21
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: *Bordetella bronchiseptica*

-continued

<400> SEQUENCE: 21

Gly Lys Pro Val Arg Arg Pro Tyr Val Asp Thr Val Ala Pro Thr Pro
 1 5 10 15

Met Lys Val Thr Ile Asp
 20

<210> SEQ ID NO 22

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 22

Gly Thr Gly Val Val Thr Val Thr
 1 5

<210> SEQ ID NO 23

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 23

Ala Ser Gly Pro Ile Val Ala Ile Ala
 1 5

<210> SEQ ID NO 24

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 24

Thr Met Val Leu Lys Val Thr Gly Ser
 1 5

<210> SEQ ID NO 25

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 25

Gly Gly Ser Leu Leu Ile Gly
 1 5

<210> SEQ ID NO 26

<211> LENGTH: 11

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 26

Val Gly Gly Ser Thr Val Thr Val Thr Phe Pro
 1 5 10

<210> SEQ ID NO 27

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 27

Arg Ala Lys Val Lys Val Asp Phe Pro
 1 5

<210> SEQ ID NO 28

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

-continued

<400> SEQUENCE: 28

Gly Gly Asp Ile Val Val Thr Gln
 1 5

<210> SEQ_ID NO 29

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 29

Gly Ala Val Arg Thr His
 1 5

<210> SEQ_ID NO 30

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 30

Leu Asp Gly Ile Val Ala Arg Phe
 1 5

<210> SEQ_ID NO 31

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 31

Gly Asp Val Val Ala Gly
 1 5

<210> SEQ_ID NO 32

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 32

Ser Gly Arg Val Thr Val Ser Gly Lys
 1 5

<210> SEQ_ID NO 33

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 33

Lys Glu Val Val Ala Gly Pro
 1 5

<210> SEQ_ID NO 34

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 34

Arg Thr Val Gln Tyr Asp
 1 5

<210> SEQ_ID NO 35

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

-continued

<400> SEQUENCE: 35

```
Phe Thr Val Ala Ser Lys Gly Asp Val
1           5
```

<210> SEQ_ID NO 36

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 36

```
Pro Ala Gly Pro Ile Arg Val Ser Ala Arg
1           5           10
```

<210> SEQ_ID NO 37

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 37

```
Asp His Tyr Leu Asp Ala
1           5
```

<210> SEQ_ID NO 38

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 38

```
Gly Ala Lys Val Arg Ile Asp
1           5
```

<210> SEQ_ID NO 39

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 39

```
Tyr Thr Val Thr Ser Thr
1           5
```

<210> SEQ_ID NO 40

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 40

```
Asp Ile Thr Val Ser Gly Thr
1           5
```

<210> SEQ_ID NO 41

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 41

```
Arg Arg Thr Val Gln Tyr Asp Asp Arg
1           5
```

<210> SEQ_ID NO 42

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

-continued

<400> SEQUENCE: 42

```

Leu Arg Pro Val Ala Arg Leu Leu Leu
1           5

```

<210> SEQ_ID NO 43

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 43

```

Ala Arg Glu Ala Thr Thr Met Val Leu
1           5

```

<210> SEQ_ID NO 44

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 44

```

Ile Arg Leu Leu Arg Pro Val Ala Arg Leu
1           5           10

```

<210> SEQ_ID NO 45

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 45

```

Ala Arg Glu Ala Thr Thr Met Val Leu Lys
1           5           10

```

<210> SEQ_ID NO 46

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 46

```

Lys Arg Pro Tyr His Asp Ile Phe Val
1           5

```

<210> SEQ_ID NO 47

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 47

```

Arg Arg Thr Val Gln Tyr Asp Asp Arg Val
1           5           10

```

<210> SEQ_ID NO 48

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 48

```

Gly Pro Val Lys Arg Pro Tyr His Asp Ile
1           5           10

```

<210> SEQ_ID NO 49

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

-continued

<400> SEQUENCE: 49

```
Glu Val Ala Thr Asp Ser Ser Ser Gly Arg
1           5           10
```

<210> SEQ_ID NO 50

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 50

```
Arg Pro Tyr His Asp Ile Phe Val Pro Val
1           5           10
```

<210> SEQ_ID NO 51

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 51

```
Ile Arg Leu Leu Arg Pro Val Ala Arg
1           5
```

<210> SEQ_ID NO 52

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 52

```
Ala Arg Phe Glu Pro Ala Asn Gly Ala
1           5
```

<210> SEQ_ID NO 53

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 53

```
Ile Arg Val Ser Ala Arg Gly Pro Arg
1           5
```

<210> SEQ_ID NO 54

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 54

```
Val Arg Ile Asp Phe Pro Asp Gly Thr Phe
1           5           10
```

<210> SEQ_ID NO 55

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 55

```
Val Pro Val Pro Pro Thr Val Glu Val
1           5
```

<210> SEQ_ID NO 56

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

-continued

<400> SEQUENCE: 56

```

Ala Pro Val Val Pro Gly Ala Asn Gly Val
1           5           10

```

<210> SEQ ID NO 57

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 57

```

Leu Glu Ser Asn Lys Met Phe Ile Tyr Leu
1           5           10

```

<210> SEQ ID NO 58

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 58

```

Gly Arg Pro Gly Asp Thr Ile Arg Val
1           5

```

<210> SEQ ID NO 59

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 59

```

Met Arg Thr Asp Gly Asn Ser Gly Val
1           5

```

<210> SEQ ID NO 60

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 60

```

Arg Arg Pro Tyr Val Asp Thr Val Ala
1           5

```

<210> SEQ ID NO 61

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 61

```

Val Arg Arg Pro Tyr Val Asp Thr Val
1           5

```

<210> SEQ ID NO 62

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 62

```

Tyr Arg Ala Thr Ser Asp Gly Asp Val
1           5

```

<210> SEQ ID NO 63

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

-continued

<400> SEQUENCE: 63

Val Arg Thr His Pro Gly Thr Gly Val
 1 5

<210> SEQ_ID NO 64

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 64

Met Arg Thr Asp Gly Asn Ser Gly Val Val
 1 5 10

<210> SEQ_ID NO 65

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 65

Val Arg Thr His Pro Gly Thr Gly Val Val
 1 5 10

<210> SEQ_ID NO 66

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 66

Asn Arg Val Pro Asn Gly Asp Tyr Pro Val
 1 5 10

<210> SEQ_ID NO 67

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 67

Ala Arg Leu Leu Ser Pro Gly Ser Met
 1 5 10

<210> SEQ_ID NO 68

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 68

Tyr Arg Leu Glu Ser Asn Lys Met Phe Ile
 1 5 10

<210> SEQ_ID NO 69

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 69

Phe Pro Gly Gly Thr Ser Lys Thr Val
 1 5

<210> SEQ_ID NO 70

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

-continued

<400> SEQUENCE: 70

Ala Pro Thr Pro Met Lys Val Thr Ile
1 5

<210> SEQ ID NO 71
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 71

Gly Pro Ser Leu Gly Gly Ser Leu Leu Ile
1 5 10

<210> SEQ ID NO 72
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 72

Ser Pro Gly Ser Met Thr Tyr Thr Glu Ile
1 5 10

<210> SEQ ID NO 73
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 73

Val Val Ala Gly Pro Asp Gly Thr Tyr Arg
1 5 10

<210> SEQ ID NO 74
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 74

Phe Pro Asp Gly Thr Thr Lys Glu Val Val
1 5 10

<210> SEQ ID NO 75
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 75

Arg Glu Ser Pro Arg Arg Thr Val Gln Tyr
1 5 10

<210> SEQ ID NO 76
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 76

Phe Pro Asp Gly Thr Thr Lys Glu Val
1 5

<210> SEQ ID NO 77
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bordetella bronchiseptica

US 9,486,514 B2

47

-continued

<400> SEQUENCE: 77

Ala Ala Leu Leu Ala Ala Ile Lys Leu
 1 5

<210> SEQ_ID NO 78

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 78

Met Pro Gly Ala Ala Gly Lys Pro Val
 1 5

<210> SEQ_ID NO 79

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 79

Phe Pro Asp Gly Thr Phe Gly Asp Val
 1 5

<210> SEQ_ID NO 80

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 80

Val Ala Pro Thr Pro Met Lys Val Thr Ile
 1 5 10

<210> SEQ_ID NO 81

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 81

Lys Leu His Asp Pro Asn Tyr Arg Leu
 1 5

<210> SEQ_ID NO 82

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 82

Asp Ala Trp Thr Lys Gln Thr Leu Leu
 1 5

<210> SEQ_ID NO 83

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

<400> SEQUENCE: 83

Asp Thr Met Asn Ser Asp Pro Tyr Asn Arg
 1 5 10

<210> SEQ_ID NO 84

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Bordetella bronchiseptica

48

-continued

<400> SEQUENCE: 84

Tyr Arg Leu Glu Ser Asn Lys Met Phe
1 5

<210> SEQ ID NO 85

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: *Bordetella bronchiseptica*

<400> SEQUENCE: 85

Gly Arg Val Thr Val Ser Gly Lys Gly Arg
1 5 10

That which is claimed is:

1. A method of producing an immune response in a mammalian subject in need thereof, comprising administering to said subject an isolated protein or peptide comprising (i) the *Bordetella* colonization factor A (BcfA) protein of SEQ ID NO: 2 or (ii) and antigenic fragments thereof comprising 20 or more contiguous amino acids thereof, in an amount effective to produce an immune response.

2. The method of claim 1, wherein said protein or peptide is an antigenic fragment of BcfA having the sequence given herein as SEQ ID NO: 3 or an antigenic fragment comprising 20 or more contiguous amino acids thereof.

3. The method of claim 1, wherein said administering further comprises an adjuvant.

4. The method of claim 1, wherein said administering delivers said BcfA protein or peptide by a mucosal route.

5. The method of claim 1, wherein said administering delivers said BcfA protein or peptide by a parenteral route.

6. The method of claim 1, wherein said administering delivers said BcfA protein or peptide by a transdermal route.

7. The method of claim 1, wherein said administering delivers said effective amount of BcfA protein or peptide in a single dose schedule.

8. The method of claim 1, wherein said administering delivers said effective amount of BcfA protein or peptide in a multiple dose schedule.

9. A method of producing an immune response in a mammalian subject in need thereof, comprising administering to said subject an isolated *Bordetella* colonization factor A (BcfA) peptide comprising an antigenic fragment of 20 or more contiguous amino acids of SEQ ID NO: 2 in an amount effective to produce an immune response.

10. The method of claim 9, wherein said administering further comprises an adjuvant.

11. The method of claim 9, wherein said administering delivers said BcfA peptide antigenic fragment by a mucosal route.

12. The method of claim 9, wherein said administering delivers said BcfA peptide antigenic fragment by a parenteral route.

13. The method of claim 9, wherein said administering delivers said BcfA peptide antigenic fragment by a transdermal route.

14. The method of claim 9, wherein said administering delivers said effective amount of BcfA peptide antigenic fragment in a single dose schedule.

15. The method of claim 9, wherein said administering delivers said effective amount of BcfA peptide antigenic fragment in a multiple dose schedule.

* * * * *